REMARKS

I. Detailed Action

Applicants acknowledge the election with traverse of Group 1, claims 1 - 22, 24 - 25 and 36. Applicants further acknowledge that foreign priority is claimed.

Applicants are submitting a corrected Figure 2 that is relabeled as 2A and 2B.

The Examiner objects to Figures 4a, 4b, 5a and 5b under 37 CFR § 1.83(a) because they fail to show the appearance of golf turf as described in the specification. It is submitted that this structural detail is not essential for a proper understanding of the invention because the description on pages 4 and 5 provides adequate information to understand the invention. For example, page 4, line 36 to page 5, line 1 clearly describes the results of rapid regrowth and greening. It is also stated on page 5, line 8 that the invention provides higher turf density. The photographs merely provide additional supporting information.

Applicants respectfully submit that in response to the objection in paragraph 7, the Applicants note that the photographs on file should be black and white photographs. This application is a PCT national phase application based on PCT/CA98/00648. At the time that this application was filed (by the prior agents of record), the PCT Office did not accept color photographs. The Applicants request that the Examiner confirm whether the photographs of record are color.

The Examienr states that the application does not contain an abstract. The application should include an abstract in the form as published on the PCT application no. PCT/CA98/00638 (shown on the cover page of the international application as published). Applicants respectfully submit an abstract as required by 37 CFR § 1.72(b) on a separate sheet. Applicants thank the

Examiner for pointing out this inadvertent mistake. Applicants further submit that amendments to the claims and the addition of new claims are fully supported by the specification. The changes do not add new matter as there is literal support for the amendments in the originally filed disclosure.

II. Claim Rejections - 35 U.S.C. § 112

Claims 4 - 6 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the claimed invention. The Examiner states that "the specification does not provide any definite evidence that overexpressing any gene involved in the synthesis of betaine or a derivative thereof, alone or in combination with cold acclimation, will result in an increase or induction of cold or freezing tolerance in the plant."

Applicants respectfully traverse this rejection. Page 10 of the description, beginning at line 34, states:

> overexpressing betaine dehydrogenase and choline monooxygenase under a low temperature promoter may allow the accumulation of betaine at the time where it could help against freezing stess.

A person skilled in the art of plant transgenics at the time the application was filed would be familiar with the techniques involved in generating a plant that expresses a gene involved in betaine synthesis under the control of a low temperature promoter.

Moreover, the description clearly demonstrates that by increasing the concentration of betaine or derivative thereof in a plant provides an increase in cold or freezing tolerance in a plant. For example, on page 10 starting at line 17:

> Betaine application early in the fall improves the performance of golf turf and consequently increased winter survival. The betaine-treated turf showed a rapid regrowth in the spring indicating a higher winter survival rate and healthier plants at spring, which have a better regrowth rate.

Also, page 8 beginning at line 9 states:

> Figs. 1A and 1B show that the survival of plants treated with betaine during cold acclimation were dramatically improved over plants that are cold acclimated in the absence of betaine.

The Applicant asserts that the particular method used to increase betaine levels in plants is irrelevant, as long as the betaine levels are increased.

Applicants further submit that researchers have successfully increased the concentration of glycine betaine by metabolic engineering. Some examples follow and it is submitted that those which were published after the Applicants' filing date provide proof that the claims were enabled as of the Applicant's filing date (ie. they confirm the inventor's predictions that betaine may be successfully overexpressed). In the examples, betaine concentration was increased by producing transgenic plants overexpressing betaine dehydrogenase and supplying exogenous betaine aldehyde (Rathinasabapathi et al., 1994, Planta 193: 155-162); by overexpressing choline monooxygenase and supplying choline (Nuccio et al., 1998, Plant J. 16: 487-496); by overexpressing choline oxidase (Sakamoto et al., 1998, Plant Mol. Biol 38: 1011-1019); and by overexpressing choline dehydrogenase and/or betaine dehydrogenase (Holmstrom et al., 2000, J. Exp. Bot. 343: 177-185). Recently, an increased betaine level was obtained by overexpressing the enzyme phosphoethanolamine N-methyltransferase in transgenic tobacco (McNeil et al., Proc. Natl Acad. Sci (U.S.A.) 2001). The overexpression of this enzyme increased the level of phosphocholine by 5 fold and free choline by 50 fold and led to a 30 fold increase in the synthesis of glycine betaine. This simple metabolic engineering using overexpression of a key

enzyme in choline and betaine synthesis supports the Applicants' prediction that manipulation of the glycine betaine pathway (or of other betaines) can be achieved by metabolic engineering.

The Examiner has cited Rathinasabapathi et al. (1994) and Nuccio et al. (1998) as a basis to allege the lack of enablement. Both of these articles relate to tobacco, which is not a plant recited in amended claim 1 (the recited plants are rosacea species, gramineae species and grass). It is therefore submitted that these references do not provide a basis to object to amended claim 1 as lacking enablement. Applicants assert that claims 4 - 6 are enabled by the specification provided.

Claims 1 - 3, 7 - 22, 24 - 25 and 36 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner states that the specification, "while being enabling for a method of increasing or inducing cold or freezing tolerance in the wheat cultivar Glenlea, does not reasonably provide enablement for other methods of increasing or inducing cold or freezing tolerance in other plants."

Applicants respectfully traverse this rejection. The Applicants assert that the application does describe how the optimal concentration of betaine can be established for several gramineae species, for example, wheat (page 8), barley (page 8 beginning at line 19), and turf grass (Figures 4 and 5; page 10 starting at line 17). Several other reports after the Applicants' filing date provide proof for the Applicants' assertion that increased betaine levels can improve cold tolerance. For example, it has been shown that an increase in betaine can improve tolerance to cold in rice (Sakamoto et al., 1998) and tobacco (Holmstrom et al., 2000). Thus, the effect of betaine accumulation in the plants recited in claim 1 is not highly unpredictable. Anyone skilled in the art could use the concentrations of betaine taught in the present application to establish the optimal concentration of betaine in new species by applying a range of concentrations.

The Examiner further cites Gibson et al. (1997) which relates to *Brassica* and a second reference, Xing et al. (2001), which relates to *Arabidopsis* as a basis to allege lack of enablement. Applicants respectfully traverse this rejection. However, in an effort to expedite prosecution, the claims now recite rosacea species, gramineae species and grasses, so it is submitted that these references do not provide a basis to object to the claims as lacking enablement. Applicants therefore respectfully request reconsideration and the withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Claims 4 - 8, 15, 18, 24 and 36 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner states that the phrase "one or more" renders the claim indefinite because it is unclear how many genes are encompassed by the claims. Applicants respectfully traverse this rejection. The Applicants submit that such language is not indefinite. Other language that is typically commonly accepted in claim drafting, such as "including a gene" can also include one or more genes because the word "including" is open ended.

Further, the Examiner states that the phrase "about an optimal cold or freezing tolerance" used in claim 36 renders the claim indefinite. Applicants respectfully traverse this rejection. However, in order to expedite prosecution claim 36 has now been amended. The claim now refers to an optimal freezing tolerance which is defined in the application as the temperature where fifty percent of a plant population die (LT_{50}).

Self-explanatory amendments or cancellations of other claims have also been made to overcome the Examiner's rejections. The amendments do not add new matter as there is literal support for the amendments in the originally filed disclosure. In light of the above amendments

and remarks, Applicants assert all the claims are now in a condition for allowance. Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

III. Claim Rejections - 35 U.S.C. § 103

Claims 1-3, 7 - 22, 24 - 25 and 36 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Rajashekar et al., in view of Kishitani et al., and in light of Zhao et al.

Applicants respectfully traverse this rejection. The results of Rajashekar et al. allegedly show that the exogenous administration of glycine betaine increased cold tolerance of leaves in both unhardened and cold-hardened strawberry plants. The Applicant submits that the results of Rajashekar et al. fail to demonstrate that the increase in freezing tolerance is observed at the whole plant level. Rajashekar et al. did not increase cold or freezing tolerance in strawberry plants but only in the leaf organ. Thus, this would not teach the successful induction of cold or freezing tolerance, because the whole plant should become tolerant, not just the leaf organ. Furthermore, there is no indication of the extent of the observed increase in freezing tolerance and importantly, Rajashekar et al. fails to show whether the increase in freezing tolerance surpasses the genotype capacity for cold acclimation and freezing tolerance. The Applicant further submits that it is impossible to predict from these results that freezing tolerance could be improved above the maximal freezing tolerance achieved by cold acclimation alone or betaine alone. Moreover, in most cases, an observation of an increase freezing tolerance at the leaf level is not indicative of a significant increase in freezing tolerance at whole plant level. The Applicants own independent tests revealed that 2 mM betaine has no effect on the freezing tolerance of strawberry or other species. Finally, the Applicants assert that if the 2 mM concentration of glycine betaine is accepted as a reference for increasing freezing tolerance in

plants, it would not have been obvious to one skilled in the art to which the present invention pertains, to use a concentration of betaine that is 125-fold higher (i.e. 250 mM).

Kishitani et al. allegedly teaches the correlation between glycine betaine levels and freezing tolerance in barley plants. The Applicant asserts that Kishitani et al. does not teach the use of exogenous betaine to provide enhanced freezing tolerance. In addition, Kishitani et al. measures the levels of endogenous glycine betaine in the plant leaves. As indicated above, an observation of an increase freezing tolerance at the leaf level is not indicative of a significant increase in freezing tolerance at whole plant level (ie. it does not indicate increased cold or freezing tolerance in the entire plant). More over, Kishitani et al. does not teach the combined treatment of exogenous betaine with cold acclimation in order to increase the level of freezing tolerance above the genetic capacity in barley plants.

Zhao et al. shows that the application of exogenous glycine betaine can act as a cryoprotectant in leaves however, the researchers fail to show the combined treatment of glycine betaine and cold acclimation to increase the level of freezing tolerance above the genetic capacity of the plant. The Applicant asserts that it would not have been obvious at the time of invention to predict that an increase in freezing tolerance of a plant above its genetic capacity can be occur with the combined treatment.

Claims 24-25 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Rajashekar et al., in view of Virtanen et al., and in light of Kishitani et al.

Applicants respectfully traverse this rejection. The Applicant respectfully disagrees with the Examiner's comments in regards to Rajashekar et al., and Kishitani et al., for the reasons stated above.

Virtanen et al. teaches the use of betaine to improve water stress tolerance and salt stress tolerance. However, Virtanen et al. fails to show the combination of cold acclimation and the exogenous administration of betaine to increase stress tolerance above the level achieved by cold acclimation or betaine used separately. The instant invention is directed to the combined effect of betaine and cold acclimation to provide a greater tolerance to freezing and to other stress. A person skilled in the art to which the current invention pertains would not have been motivated to perform this combined treatment because it was generally believed that plant tolerance was dictated by the genotype capacity of a plant. The finding that one could increase tolerance above the known genotypic capacity to acclimate was both surprising and unexpected. Applicants strongly assert that the cited references do not teach, nor suggest, the claimed unique invention. Applicants respectfully request reconsideration and withdrawal of the objections under 35 U.S.C. § 103(a).

IV. Conclusion

In light of the above amendments and remarks, Applicants assert that the claims as amended are in a condition for allowance. Applicants respectfully request reconsideration and withdrawal of the above rejections to claims 1 - 22, 24, 25 and 36.

Please consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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Application No. 09/446,711

AMENDMENT — VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Please add the following abstract as page 17 to the patent application.

ABSTRACT

The accumulation of the osmolyte glycine-betaine provides increased freezing tolerance (FT) in wheat. An exogenous application of betaine at room temperature increased FT by more than 5 degrees Celsius. The treated plants showed some of the characteristics of cold-acclimated plants and expressed the low temperature responsive gene *Wcor410* encoding a major membrane associated protein. The combined exposure to low temperature and betaine resulted in a cumulative effect on the improvement of FT which surpassed the genetic potential of the plant to withstand freezing. This result was demonstrated in wheat and barley under controlled environments and under field conditions for two grass species of golf turf. This important finding shows that an exogenous application of betaine before a predicted frost is usefully exploited to substantially improve cold or freezing tolerance in gramineae species, grasses, and other plants. This physiological treatment improves the genotype capacity to cold acclimate and acquire superior freezing tolerance without modifying the genetic background. These results show that manipulating endogenous betaine levels by any means should result in a similar improvement.

In the Claims

Please cancel claims 5 - 8, 15, 23, 25 - 35 and 37.

Please amend claims 1, 4, 16 - 20, 22, 24 and 36 as follows:

1. (Amended)

A method of increasing or inducing cold or freezing tolerance in [a plant] rosacea species plant, gramineae species plant or grass plant, which comprises the following steps:

a) acclimating said plant to a temperature not lower than the coldest temperature that said plant is capable of withstanding, for a time sufficient to induce cold or freezing tolerance in said plant, and

b) increasing the concentration of betaine or a derivative thereof to a non toxic concentration in said plant to induce cold or freezing tolerance in said plant;

whereby combined steps a) and b) increase or induce cold or freezing tolerance of said plant over and above the cold or freezing tolerance of the normal genotypic potential induced by each step alone.

4. (Amended)

A method as set forth in claim 1, wherein the step of increasing the concentration of betaine or a derivative thereof includes overexpressing one or more genes involved in the synthesis of betaine or a derivative thereof selected from the group consisting of betaine dehydrogenase and choline monooxygenase.

16. (Amended)

A method as set forth in claim [15] 1, wherein said plant is selected from the group consisting of roses, strawberry, golf turf, barley and wheat.

17. (Amended)

A method as set forth in claim [15] 1, wherein said plant comprises golf turf.

18. (Amended)

A method as set forth in claim [10 or 11] 3, wherein said composition comprises glycine betaine at a concentration lower than about 500mM.

19. (Amended)

A method as set forth in claim 12, wherein said glycine betaine is present at a concentration [is] of about 250 mM.

20. (Amended)

A method as set forth in claim 13, wherein said compound is present at a concentration [is] of about 250 mM.

22. (Amended)

A method as set forth in claim 20, wherein the increase in freezing tolerance is by at least about [5°C] 6°C.

24. (Amended)

A method as set forth in claim 1, which further results in improving [photosynthetic capacity and overall physiology of said plant] regrowth, greening and resistance to photoinhibition of said rosacea species, gramineae species and grasses at cold [temperature] or freezing temperatures of about 6°C to about -17°C.

36. (Amended)

A method as set forth in claim 1 [any one of claims 1, 33 and 34], wherein [each mention of] said [cold or] freezing tolerance comprises about an optimal [cold or] freezing tolerance.

Please add the following new claims:

38. (New)

A method as set forth in claim 1, wherein said acclimation temperature and said coldest temperature that said rosacea species, gramineae species and grasses are capable of withstanding is between 6°C to about -17°C.

39. (New)

A method as set forth in claim 1, wherein said gramineae species and grasses are selected from a group of gramineae species and grasses sensitive to temperatures between 6°C to about -17°C.

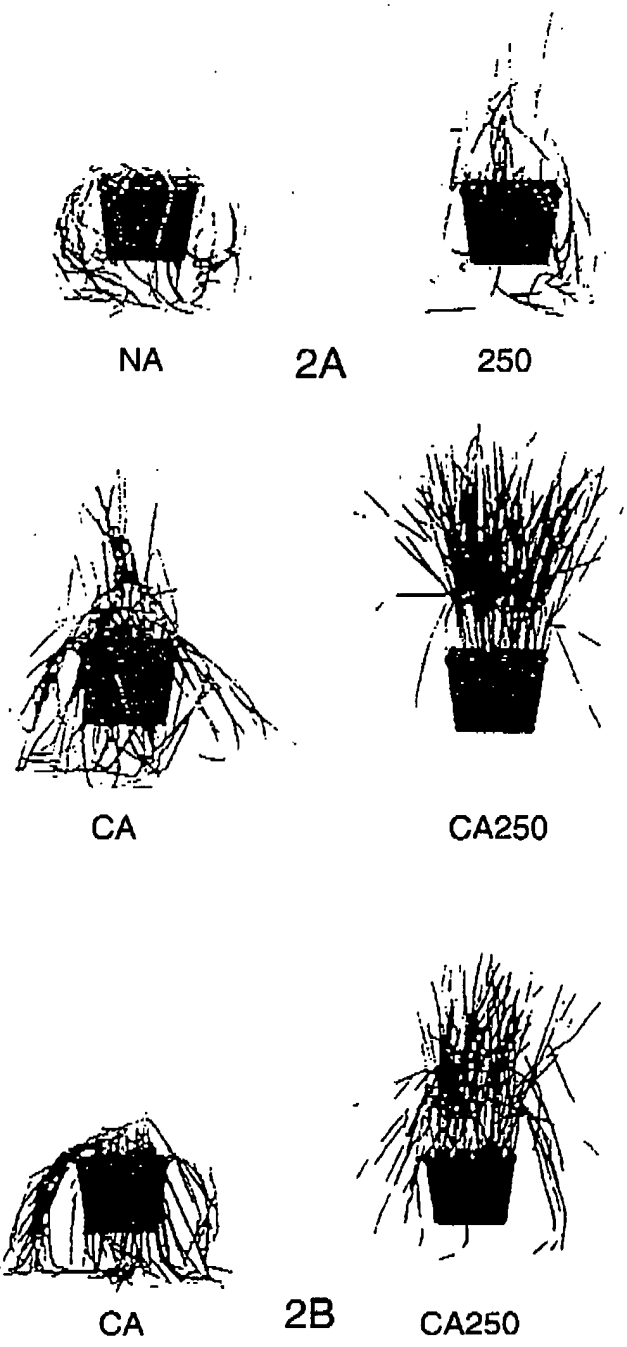

FIG. 2